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TRIGONELLINE AND PROMOTION OF CELL ARREST IN G2 OF VARIOUS LEGUMES

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Key Word Index—*Pisum sativum*; *Glycine max*; *Phaseolus vulgaris*; Leguminosae; trigonelline; cell arrest.

Abstract—Trigonelline, present in dry seeds of *Pisum sativum*, is transported to enlarging roots and shoots during early seedling ontogeny and promotes cell arrest in G2 in 40% of all root cells. In the absence of trigonelline, this cell population arrests in G1. Results presented herein show that trigonelline also promotes cell arrest in G2 in roots of *Glycine max* and *Phaseolus vulgaris* and that the percentage of cells that arrest in G2 in roots of *G. max* decreases during seedling ontogeny, as it does in *P. sativum*. During development, trigonelline is synthesized in leaves and is translocated to pods and eventually to seeds during fruit maturation in *P. sativum* and *G. max*. Seeds of most legumes have high concentrations of trigonelline and those of some non-legumes have low concentrations.

INTRODUCTION

Trigonelline functions as a plant hormone in *Pisum sativum* [1–3]. Trigonelline, found in cotyledons of dry seeds, is transported from cotyledons to other plant tissues during early seedling development and promotes preferential cell arrest in G2 of the cell cycle.

Arrest in the cell cycle of meristematic cells in cultured primary root tips after temporary carbohydrate deprivation (to produce stationary phase meristems) is a non-random process [4]. Cell arrest during normal cell differentiation (e.g. in more mature root segments) also occurs as a non-random process. Results have also shown that the proportions of cells with 2C and 4C DNA contents in stationary phase meristems are similar to the proportions in more mature root segments [5]. Few cells arrest during DNA synthesis and no cells arrest during mitosis. Under temporary carbohydrate deprivation, root cells of *Pisum sativum* arrest in G1, in G2, or become polyploid. If trigonelline is present, up to 60% of the meristem cell population arrests in G2. If sufficient trigonelline is not present, most cells arrest only in G1 [2, 4, 6].

Trigonelline has been detected in many plants and animals. In plant cell cultures, it has been found in several gymnosperms and in several monocotyledonous and many dicotyledonous plants [7]. Moreover, trigonelline has been found in a wide variety of animals [8]. Many mammals excrete trigonelline in urine [9]. No hormone effects of trigonelline have been found in animals.

Trigonelline is usually the most abundant molecule in the pyridine nucleotide metabolic pathway for the production of NAD [10–12]. These results indicate that trigonelline may have a regulatory role in NAD biosynthesis as well as in cell proliferation.

Experiments reported herein were performed to determine: (1) if trigonelline promotes cell arrest in G2 in other legumes besides *P. sativum*; (2) if the proportion of cells arrested in G2 decreases in soybean roots as a function of seedling age, as it does in roots of *P. sativum*; (3) trigonelline concentrations in various tissues of peas and soybeans during ontogeny; and (4) concentrations of trigonelline in dry seeds of various plant species.

RESULTS

Since most of the previous research to demonstrate that trigonelline is a hormone that influences cell cycle kinetics of roots and shoots was done with *P. sativum*, experiments were designed to determine if trigonelline had similar effects in two other legumes. When excised roots of *G. max* and *P. vulgaris* (Table 1) were exposed to trigonelline, preferential cell arrest in G2 was evident. A concentration of 10^{-4} M trigonelline in the bioassay increased the proportion of cells arrested in G2 by 0.14 and 0.12 in *G. max* and *P. vulgaris*, respectively.

Since trigonelline promoted preferential cell arrest in G2 of *G. max* and *P. vulgaris* in aseptic culture of excised roots, experiments were performed to determine if the proportion of cells arrested in G2 in *G. max* naturally decreases during seedling ontogeny as it does in *P. sativum* [3]. When excised roots from 3-, 5-, 7- and 10-day-old plants of *G. max* were placed in medium without sucrose for 3 days to establish a stationary phase, the proportions of cells arrested in G2 were 0.50, 0.34, 0.17 and 0.11, respectively. The proportion of cells arrested in G2 decreased during early seedling ontogeny.

Previous results suggested that the total amount of trigonelline in seedlings of *P. sativum* is relatively constant during the first 7 days after germination and that the amount increases by *ca* 25% in 10-day-old seedlings [13]. Experiments were performed to determine the concentration of this hormone during plant development (Table 2). As seedling age increased, trigonelline concentrations decreased in roots and stems to 1 μg/g tissue or lower. From 20 to 60 days, the concentration of trigonelline in leaves decreased from 88 to 8.8 μg/g tissue. In 40-day-old plants, a high trigonelline concentration (203 μg/g tissue) was present in pods but most of this trigonelline was present in seeds as the plants matured. At

Table 1. Demonstration that trigonelline promotes perferential cell arrest in G2 stationary phase meristems of soybeans (*Glycine max*) and pinto beans (*Phaseolus vulgaris*)

Treatment	Proportion of cells in G2	
	Soybeans	Pinto beans
3-Day-old seedlings—excised roots placed in medium without carbohydrate for 4 days	0.50±0.08	0.41±0.04
3-Day-old seedlings—excised roots grown in medium with sucrose without trigonelline before placement in medium without sucrose for 4 days	0.18±0.03	0.18±0.03
3-Day-old seedlings—excised roots grown in medium with sucrose and 10^{-4} M trigonelline before placement in medium without sucrose for 4 days	0.32±0.02	0.30±0.03

Values represent mean and standard error, respectively.

maturity, most of the trigonelline in the entire plant was present in the seeds.

Since the response of the roots of *G. max* and of *P. sativum* to trigonelline is similar, experiments were performed to determine trigonelline concentrations in various organs of plants of *G. max*. Concentrations of trigonelline were below 1.5 μg/g tissue in roots throughout ontogeny. Trigonelline concentrations were below 0.5 μg/g in stem tissues from 20 to 60 days. A much higher level (7.6 μg/g tissue) was present in 70-day-old stem tissues but the concentration decreased thereafter (Table 3). Trigonelline concentrations were relatively high (63 μg/g tissue) in leaves of 20-day-old plants but decreased gradually so that 100-day-old plants had only *ca* 10 μg/g tissue. Trigonelline concentrations in pods of 60- to 70-day-old plants were *ca* 15 μg/g tissue but decreased to *ca* 4 μg/g as seeds matured. Seeds had relatively constant concentrations of *ca* 20 μg/g fresh tissue.

These results show that roots of *G. max* and *P. vulgaris* exhibited promotion of cell arrest in G2 with added trigonelline similar to that of *P. sativum*. Moreover, roots of *Helianthus annuus* and *Triticum aestivum* do not respond to trigonelline [14]. To determine if trigonelline may have a hormonal role in several other plant species, assays were performed to determine trigonelline concentrations of several plant species (Table 4). Except for *Arachis hypogea* (peanut), all the legumes tested had relatively high trigonelline concentrations. In contrast, most non-legumes and monocots had relatively low trigonelline concentrations in dry seeds.

DISCUSSION

Trigonelline has been shown to promote cell arrest in G2 in roots of four legumes, *Pisum sativum*, *Vicia faba*, *Glycine max* and *Phaseolus vulgaris*. It does not seem to influence the two non-legumes tested (*Triticum aestivum* and *Helianthus annuus*) [14]. It seems that trigonelline affects many legumes by promoting cell arrest in G2. The reason(s) for this apparent specificity is unknown.

Predominant cell arrest in G2 in roots is common within but is not restricted to legumes. Several other non-legumes such as *Allium cepa* [15], *Zea mays* [16] and *Raphanus sativus* [17] exhibit cell arrest in G2 in mature roots and these should be tested to determine if they respond to trigonelline, since both *T. aestivum* and *H. annuus* show cell arrest mainly in G1 [5].

Trigonelline concentrations are high in seeds of most of the legumes tested and are markedly lower in some non-legumes. However, it cannot be concluded that high trigonelline concentrations are restricted only to legumes. Seeds of *Coffea arabica* (Rubiaceae) have large concentrations of trigonelline [18]. Also, seeds of *Secale cereale* (rye) contain (52 μg/g tissue) trigonelline.

Trigonelline contents in various plant organs changed during ontogeny in the two legumes tested. In general, the concentration in young seedlings is the same as that in dry seeds suggesting that trigonelline is not synthesized and/or destroyed during that time period. After seedling establishment, trigonelline seems to be synthesized in leaves. It is transported to pods and eventually to developing seeds.

EXPERIMENTAL

General culture conditions. Seeds of *Pisum sativum* L. cv Alaska 2B; *Glycine max* Merrill cv Amsoy 71 and *Phaseolus vulgaris* L. cv

Table 2. Trigonelline concentrations in various tissues of *Pisum sativum* during ontogeny

Plant age (days)	Trigonelline concentration (μg/g fresh mass)				
	Roots	Shoots	Leaves	Pods	Seeds
20	6.3±0.2	24.2±5.3	88.2±6.0	—	—
30	6.7±3.0	19.8±1.0	28.0±0.0	—	—
40	0.7±0.2	3.5±0.2	11.1±3.0	203±4*	—
50	†	1.2±0.5	20.2±0.0	37.7±7.0	128±2
60	1.0±0.6	†	8.8±4.6	5.8±4.8	166±16

*Immature seeds were not separated from the pods at this age because seeds were very small and would have had an insignificant mass compared with the mass of the pods. Values represent mean and standard error, respectively.

†Samples below 0.5 μg/g tissue, which is the lowest limit of detection of trigonelline with the procedures used.

Table 3. Trigonelline concentrations in various fresh tissues of *Glycine max* during ontogeny

Plant age (days)	Trigonelline concentration (μg/g fresh mass)				
	Roots	Stems	Leaves	Pods	Seeds
20	*	*	63.2±4.4	—	—
30	*	*	40.3±5.6	—	—
40	*	*	48.8±2.2	—	—
50	*	*	27.8±2.3	—	—
60	*	*	39.4±2.0	16.2±3.2†	—
70	*	7.6±1.0	19.5±1.9	14.8±1.4†	—
80	1.1±0.05	1.5±1.0	18.3±11.6	5.3±0.4†	—
90	*	2.0±1.0	10.8±0.4	4.5±0.5	19.7±2.6
100	*	3.0±0.5	10.5±4.5	3.7±2.0	21.7±3.3

*Samples below 0.5 μg/g tissue, which is the lowest limit of detection of trigonelline with the procedures used.

†Immature seeds were not separated from the pods at this point because seeds were very small and would have had an insignificant mass compared with the mass of the pods. Values represent mean and standard error, respectively.

Table 4. Trigonelline concentrations in dry seeds of various plant species

Plant species	Trigonelline concentration (μg/g tissue)
Dicotyledonous plants	
Legumes	
Pisum sativum	227±11
Vicia faba	294±14
Glycine max	71.8±5
Phaseolus vulgaris (pinto bean)	344±17
Phaseolus vulgaris (white bean)	554±13
Arachis hypogea (peanut)	8.6±3
Lens culinaris (lentil)	363±18
Psophocarpus tetragonolobus (winged beans)	384±19
Non-legume	
Raphanus sativus (radish)	7.6±2.1
Solanum melongena (egg-plant)	1.1±0.0
Cucurbita maxima (squash)	1.8±0.5
Lactuca sativa (lettuce)	11.7±2.0
Capsicum annum (pepper)	0.60±0.0
Cucumis melo (muskmelon)	2.6±0.0
Monocotyledonous plants	
Hordeum vulgare (barley)	89±4.0
Triticum aestivum (wheat)	*
Zea mays (maize)	4.0±2.0
Secale cereale (rye)	52.0±2.6
Sorghum halepense (sorghum)	14.9±4.9
Allium cepa (onion)	13.0±2.0

*Sample below 0.5 μg/g tissue, which is the lowest limit of detection of trigonelline with the procedures used. Values represent mean and standard error, respectively.

Univ. of Idaho 111 were surface-sterilized for 10 min with undiluted Clorox^{R} (containing 5.25% Na hypochlorite), washed with sterile H_2O to remove bleach, and germinated in sterile vermiculite.

Bioassay for promotion of cell arrest in G2. A bioassay was used to determine the ability of trigonelline to promote cell arrest in G2 [4]. Under aseptic conditions, excised root meristems from 3-day-old seedlings were placed in culture medium with sucrose for 3 days before temporary carbohydrate deprivation (establishment of a stationary phase meristem). To establish stationary phase meristems, roots were placed in culture medium for 3 days. A stationary phase meristem may be defined as a meristem (0–2 mm portion) in which progression through the cell cycle has ceased temporarily [5]. In some experiments trigonelline (Sigma) was added to the sucrose medium which normally supports growth of 10 excised roots. After eventual establishment of a stationary phase by temporary carbohydrate deprivation, cells were arrested either in G1 or in G2 (2C and 4C contents, respectively) within the terminal meristem. For example, when roots from 3-day-old seedlings were placed in sucrose medium alone before establishment of the stationary phase, only 0.20 cells arrested in G2. However, if a sufficient concn of trigonelline was present in the medium with sucrose before establishment of the stationary phase, a larger proportion (≈ 0.40–0.60) of cells would arrest in G2 [2]. The stationary phase technique was used to determine if root meristems of *G. max* and *P. vulgaris* respond to trigonelline.

DNA measurements. Measurements of relative DNA per nucleus of Feulgen-stained nuclei were obtained by microfluorimetry [4, 19].

Intact plant studies. Garden pea and soybean plants were grown to maturity in a soil mixture which consisted of 40% topsoil, 40% perlite, 20% peat, buffered with lime. The appropriate *Rhizobium* inoculum was also added. Plants were sacrificed every 10 days so that various organs and tissues could be extracted to determine trigonelline concn.

Trigonelline concentrations. To determine concns of trigonelline, all tissues from plants at each age were separated into roots, stems, leaves, pods and seeds, and extracted in an EtOH series [19]. Each extract was concd and spotted on silica gel TLC UV plates, 250 μm thick (Analtech). Extracts were developed in Me_2CO–H_2O (1:1). Plates were allowed to dry in air and trigonelline was eluted. Quantities of trigonelline were determined by HPLC [20] using a Whatman Partisil-Sax 10 column using 7 mM KPi as solvent (pH 5.8) at a flow rate of

1 ml/min. Recovery of trigonelline from TLC plates was usually *ca* 50%. For each experiment, authentic trigonelline (Sigma) was applied to the TLC plates, eluted and processed like plant extracts through HPLC. Plant samples were corrected by the recovery percentage for each experiment.

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